PHILIPPINE SCIENCE HIGH SCHOOL WESTERN VISAYAS
Doña Lawaan H. Lopez Campus
Iloilo City

DERMAL OINTMENT FROM GINGER ROOT (*Zingiber officionale*) AND BETEL LEAF (*Piper betle*) AGAINST COMMON SKIN INFECTIONS

A Research Paper Presented to the
Faculty of the Philippine Science High School Western Visayas
Iloilo City

In Partial Fulfillment
of the Requirements in
Technology Research II

By

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Johnraja-Zaid M. Ladja
Mark Paul T. Lomod

February 2000

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APPROVAL SHEET

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Abstract

This study aimed to determine the feasibility of dermal ointment using ginger (*Zingiber officinale*) and betel leaf (*Piper betle*) essential oils as the main ingredients. It also aimed to determine the effectiveness of the ointment produced against *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris*. Furthermore, it aimed to determine the most effective treatment concentrations in terms of the zones of inhibition they caused upon the test bacteria, and whether their effectiveness showed a significant difference among each other. It was hypothesized that there is no significant difference in the effectiveness of the three ointment treatment concentrations, namely: 70% Ginger-0% Betel-30% Petroleum Jelly, 35% Ginger-35% Betel-30% Petroleum Jelly, and 0% Ginger-70% Betel-30% Petroleum Jelly in terms of the zones of inhibition they caused upon the test bacteria. The independent variables of this study were the different concentrations of ginger and betel leaf oils, while the dependent variables were the zones of inhibition of the treatments upon the

three test bacteria. The positive and negative test controls were Dermalin, a commercial ointment, and ether, the organic solvent used to extract the essential oils from the crude extracts, respectively. The study was performed in the Science Research Laboratory at the Philippine Science High School Western Visayas Campus. Four replicates were prepared for greater accuracy in the analysis of data. The One-way Analysis of Variance (ANOVA), set at 0.05 level of significance, was employed as the inferential statistical tool. The Scheffe test, also set at 0.05 level of significance, was used as the post hoc multiple comparison test.

This study revealed that ginger root and betel leaf essential oils, when used as the main ingredients in the production of dermal ointment, was feasible. It was noted in the case of *Staphylococcus aureus*, where the Ginger-Betel treatment had the highest diameter zone of inhibition, that the two ointment treatments' effectiveness increased as both were combined rather than each working independently. In the two other bacteria, Dermalin still topped. Nevertheless, tests showed that the antibacterial effect of the three ointment treatments did not vary significantly with each other, or with that of the positive control, showing that the four treatments

can be substituted for each other without affecting their observed degree of effectiveness.

TABLE OF CONTENTS

List of Tables

List of Figures

PHILIPPINE SCIENCE HIGH SCHOOL WESTERN VISAYAS
Doña Lawaan H. Lopez Campus
Iloilo City

DERMAL OINTMENT FROM GINGER ROOT (*Zingiber officionale*) AND BETEL LEAF (*Piper betle*) AGAINST COMMON SKIN INFECTIONS

Chapter 1

Introduction to the Study

Background of the Study

Ever since the beginning of man's existence, disease has always been there, even perhaps before then. In the past and various times, man has always learned to cope well with the different kinds of manifestations of disease through his knowledge of the medicinal properties in nature, and because of this knowledge technology stepped in. Various plant extracts found to have medicinal/antibacterial properties were mass produced and made accessible. Unfortunately, with the increase in demand and continuous scarcity of raw materials, the price of these medicines catapulted.

Alarmed at this slow yet definite turn of events, a going back to the basics-- discover cheaper (and if possible, more effective) alternatives for the very popular yet inaccessible commercial brands. We turned to plants that were a household

name but for their numerous possible medicinal uses. The fact that these plants were easy to obtain was an added advantage.

The presence of antibacterial properties in Philippine local plants had been evident in reports of scientists who have listed a number of plant species commonly used by quack doctors and herbolarios for the treatment of various infectious diseases in rural areas (Caverte and Orbina, 1997).

It was also interesting to note that most treatments utilized by quack doctors rely on the direct application of the ground parts of these plants to the afflicted area. In short, although the applied plant extracts were extracted crudely, most treatments were effective nevertheless. Because of this observation, it was surmised that probably one of the most common disorders afflicting the ordinary rural person who cannot afford to buy the expensive commercial medication are skin infections. Also, because skin infections are diseases which are more obvious to the eye and thus may not only cause physical pain and discomfort to the sick person, but also a great amount of emotional shame. This became all the more reason to focus the study on skin diseases, the most common bacteria which cause skin infections and the possible antibacterial property of two local plants.

The study was based on the previous work done by Caverte and Orbina (1997) which focused on the extraction of essential oils from an array of local plants, and the separate testing of each acquired extract on different strains of pathogenic bacteria through the agar plate method and the mean diameter zone of inhibition. Their results showed betel leaf (*Piper betle*) and ginger (*Zingiber officinale*) to have the highest antibacterial property.

Their conclusion gave the researchers an idea to combine the extracts of these two plants and determine if the resulting combination would be more effective against the bacterial specimens, or if both would work well independently.

Finally, it was thought about the making of the extracts into something readily useful, thus a dermal ointment came to mind.

The relationship between the independent and dependent variable is presented in Figure 1.

INDEPENDENT VARIABLE DEPENDENT VARIABLE

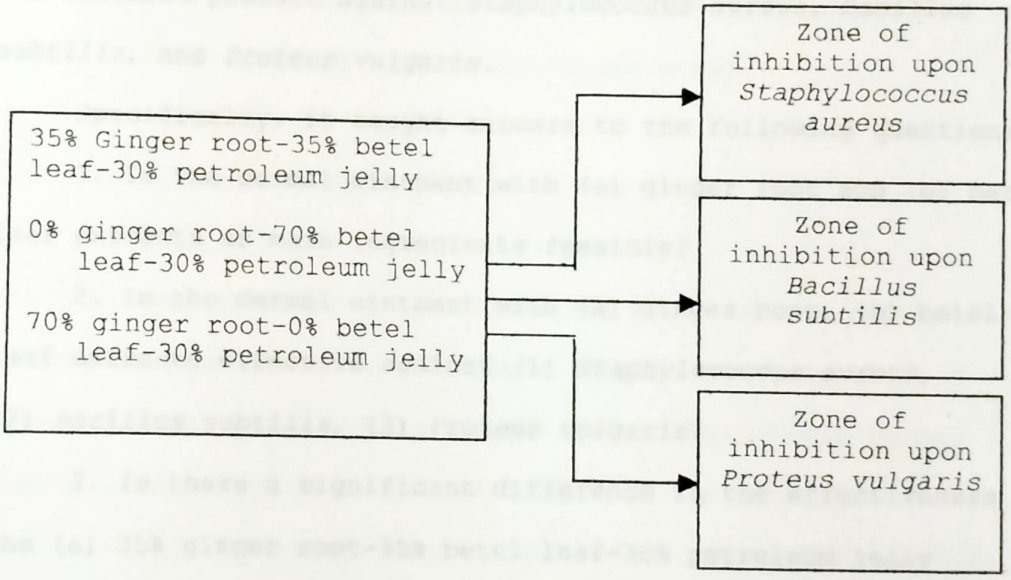

Figure 1. The effects of the three essential oil concentrations upon the zones of inhibition *Staphylococcus aureus, Bacillus subtilis, and Proteus vulgaris*

Statement of the Problem and the Hypothesis

This study aimed to determine the feasibility of dermal ointment using ginger (*Zingiber officinale*) root and betel (*Piper betle*) essential oils as the main ingredients.

Furthermore, it aimed to determine the effectiveness of the ointment product against *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*.

Specifically, it sought answers to the following questions:

1. Is the dermal ointment with (a) ginger root and (b) betel leaf extracts as main ingredients feasible?

2. Is the dermal ointment with (a) ginger root, (b) betel leaf extracts effective against (1) *Staphylococcus aureus*, (2) *Bacillus subtilis*, (3) *Proteus vulgaris*?

3. Is there a significant difference in the effectiveness of the (a) 35% ginger root-35% betel leaf-30% petroleum jelly (b) 0% ginger root-70% betel leaf-30% petroleum jelly and, (c) 70% ginger root-0% betel leaf-30% petroleum jelly treatments effective in terms of the zones of inhibition they cause upon (1) *Staphylococcus aureus*, (2) *Bacillus subtilis* and (3) *Proteus vulgaris*?

Based on the problems given, it was hypothesized that there is no significant difference in the effectiveness of (a) 35% ginger root-35% betel leaf-30% petroleum jelly (b) 0% ginger root-70% betel leaf-30% petroleum jelly and, (c) 70% ginger root-0% betel leaf-30% petroleum jelly treatments in terms of the zones of inhibition they caused upon (1) *Staphylococcus aureus*, (2) *Bacillus subtilis* and (3) *Proteus vulgaris* when compared with Dermalin and ether.

Significance of the Study

Brought about by the skyrocketing price of laboratory produced drugs nowadays, we thought of manufacturing an affordable and easy-to-make dermal ointment utilizing common plants like ginger root and betel leaf as the primary ingredients. This way, many Filipinos can attain treatment for their minor skin afflictions without the need of buying commercial drugs that are beyond their family budgets.

Furthermore, the research work might serve as a reference, which can be used by future researchers pursuing studies on related or similar topics.

Definition of Terms

For the purpose of clarity, the following terms were hereby given their corresponding conceptual and operational definitions:

Ginger root- is the spice made from the rhizome, or enlarged underground stem of the herbaceous perennial plant *Zingiber officinale*, a member of the ginger family, Zingiberaceae (The New Lexicon Webster's Encyclopedic Dictionary, 1992).

In this study, essential oils from ginger root were used as one of the ingredients in producing the ointment.

Betel leaf- a diocious smooth climbing vine reaching a height of 2 to 4 meters, the leaves of which are used as a masticatory (Quisumbing, 1978)

In this study, essential oils from betel leaf were used as one of the ingredients in producing the ointment.

Extract- is a concentrated essence, used especially for flavoring; the solid or semi-solid matter which remains of a substance after evaporation of moisture or the use of solvents (The New Lexicon Webster's Encyclopedic Dictionary, 1992).

In this study, the term referred to the essential oil taken from the ginger root and betel leaf.

Ointment- is a semisolid, usually greasy and medicated preparation for application to the skin (The New Lexicon Webster's Encyclopedic Dictionary).

In this study, the term meant the mixture of petroleum jelly and the plant extracts in different proportions.

Staphylococcus aureus- is a Gram-positive spherical bacterium found on the skin and nostrils of many healthy individuals. These bacteria often give rise to minor superficial diseases including the formation of pustules or boils in hair follicles characterized by the presence of pus and formation of abscesses, skin pustules, boils and carbuncles (Microsoft Encarta 98 Encyclopedia, 1998).

In this study, this bacterial species was used as one of the three test organisms.

Bacillus subtilis- are spore-forming rod shaped bacteria. Colonies are irregular and have a curled or hair-like structure or sometimes called Medusa head (Basic Microbiology, 1992).

In this study, this bacterial species was used as one of the three test organisms.

Proteus vulgaris- is a motile, non-lactose fermenter found in feces, sewage and soil, which causes a number of opportunistic infections (Basic Microbiology).

In this study, this bacterial specie was used as one of the three test organisms.

Scope and Delimitation of the Study

This study considered only the use of Ginger root and Betel leaf as the sources of essential oils for the ointment treatments. The test organisms were *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus vulgaris*. The essential oil concentrations are 70% Ginger-0% Betel-30% Petroleum Jelly, 35% Ginger-35% Betel-30% Petroleum Jelly, and 0% Ginger-70% Betel-30% Petroleum Jelly.

Formation of the product and subsequent testing were done at the PSHSWV Research Laboratory.

Chapter 2

Review of Related Literature

Essential Oils

Essential oils are odorous, volatile, usually liquid, substances occurring in certain plant species or only in parts of the plant, as the flowers, fruit, seed, leaves, bark, wood or the root. In fact, the essential oil is responsible for the characteristic odor and flavor of these plants or plant parts. Unlike the fatty oils, such as olive, cottonseed, or almond oil, the essential oils evaporate at room temperature.

There are some 200 commercially produced essential oils, all obtained from the oil-bearing leaves, flowers, bark, seeds, or wood of aromatic plants. Steam distillation is the most common method of extraction, particularly for oils from seeds or bark. In this process, hot steam is passed over the plant tissues, and the volatile compounds evaporate and are then condensed in water. Citrus oils are expressed from the flavor sacs in the rind of the fruits. Some flower petals, whose oils are destroyed by steam processing, are treated by the slow process of enfleurage: the blossoms are placed on a layer of cold fat, which absorbs the oil; fresh blossoms are added until the fat is completely oil

saturated; and the resulting substance, called a pomade is washed with alcohol to remove the oil.

All essential oils are soluble in alcohol, and flavoring extracts, such as oil of cloves, vanilla, and the other oils from citrus fruits, are usually sold as alcohol solution (Grolier International Encyclopedia, 1991).

Essential oils from some Philippine plants had been cited by some researchers for positive results of their efficacy against pathogenic bacteria. Essential oils are aromatic substances produced by certain plants. Oftentimes, they are used as perfume scents or food flavorings. Chemically complex, essential oils are a mixture of organic compounds, primarily terpenes (Grolier Multimedia Encyclopedia, 1993).

Ginger (*Zingiber officinale*)

Ginger is an erect, smooth plant rising from thickened, very aromatic rootstocks. The leafy stems are 0.4 to 1 meter high. The leaves are distichous, lanceolate to linear-lanceolate, 15 to 25 centimeters long and 2 centimeters wide or less. The scape rising from the rootstocks is erect, 15 to 25 centimeters high, and covered with distant, imbricate bracts.

The spike is ovoid to ellipsoid, and about 5 centimeters long. The bracts are ovate, cupsidate and about 2.5 centimeters long or somewhat less. The corolla is greenish yellow, and its tube is less than 2 centimeters long; while the lip is oblong-obovate and slightly purplish.

Ginger contains the following constituents: an aromatic volatile oil (0.25- 3 percent) containing camphene, phellandrene, zingiberene, cineol, and borneol; gingerol, a yellow pungent body; an oleo-resin, gingerin, the active principle; other resins; and starch. It also contains singerone, zingiberole, citral, linalool, geraniol, chavicol, vanillyl alcohol, caprylic acid, methyl heptenon, pelargon-aldehyde and malate.

It is widely cultivated although not on an extensive scale and is nowhere naturalized. It is a native of tropical Asia is now pantropic in cultivation.

For a tropical plant, ginger is surprisingly adaptable: it can be grown anywhere except where summers are short and cool. Where summer is warm and lasts for five to eight months, ginger will produce new rhizomes, but no seeds. The tops will usually die down at the end of the season.

Gingerroot's name comes from the Sanskrit word for "horn root", undoubtedly referring to its knobby appearance.

The rhizomes of ginger are used as a condiment, being one of the most popular flavoring agents known. Ginger ale and ginger beer, also made from the rhizomes, are refreshing drinks. Tahu, or salabat, a native popular beverage, is also prepared from the rhizomes. The pungency is due to the pungent principle, mainly zingerone and shogaol.

Ginger is extremely valuable in dyspepsia, flatulence, colic, vomiting, spasms and other painful affections of the stomach and the bowels unattended by fever. It is also very effective for colds, coughs, asthma, dyspepsia, and indigestion. Ginger taken with rock-salt before meals is said to clean the tongue and throat, increase the appetite, and produce an agreeable sensation. People suffering from biliousness and delirium, relaxed sorethroat, hoarseness and loss of voice are sometimes benefited by chewing a piece of ginger, thus producing a copious flow of saliva.

Dry ginger is generally used as corrective adjunct to purgatives to prevent nausea and griping. The juice is expressed from fresh ginger in gradually increasing doses is a strong diuretic in cases of general dropsy.

In chronic rheumatism an infusion of ginger (2 grams to 6 ounces of boiling water, and strained), taken warm the last thing before going to bed, the body being covered with blankets so as to produce copious perspiration, is often attended with the best effects. The same treatment has also been found very beneficial in colds or catarrhal attacks, and during the cold stages of intermittent fevers. For headaches a ginger plaster, made by bruising ginger with a little water to the consistence of a poultice, and applied to the forehead, affords in many instances much relief. Toothache and faceache are sometimes relieved by the same poultice applied to the face. A hot infusion is very useful for the stoppage of the menses due to cold. The rhizomes are prescribed for tuberculosis, general fatigue, and affections of the uterus.

Ginger also helps promote gastric secretions, thus aiding with food absorption. It is excellent for indigestion, flatulence, nausea (including morning and travel sickness), and colic. It is stimulating to the circulation and will help to warm cold hands and feet. It has a beneficial effect on the lungs, helping to dispel mucus and phlegm. Taken hot, it promotes sweating and is helpful for colds and flus. It is also

used for suppressed menstruation. Chewing the root will stimulate the saliva and benefit the sore throat.

In the East and Malaya, fresh ginger plays an important part in curry.

As an external medicine, the Filipinos use the pounded rhizome alone or mixed with oil as a revulsive and antirheumatic. Internally it is used in decoction as a stomachic and stimulant, especially in flatulence and colic. Ginger juice rubbed on and around the navel is said to cure all kinds of diarrhea. The rhizome is also used as rubefacient.

In Indo-China, a cataplasm is good for furuncles, and, when mixed with oil, is antirheumatic. The action of the drug is as stomachic, carminative, stimulant, diaphoritic, sialogogue, and digestive.

In India, dry ginger is much used as a carminative adjunct along with black pepper and long pepper.

In Perak, the rhizome is a well-known vermifuge. In the Antilles powdered rhizome is prescribed as a revulsive for pleurisis (Quisumbing, 1978).

Ginger is also a well-known spice used in food and has for centuries, been an essential ingredient of traditional Chinese medicines (http://hne-jpn.com/, 1998).

The Chinese use the fresh ginger root for treatment of colds and for sweating, and the dried root for respiratory and digestive disorders. Its other uses include: relief of headaches, aches and pains, and as a cleansing agent through the kidneys and bowels.

Pounded leaves are applied as a warm poultice to bruises. As an external medicine, the pounded rhizome alone or mixed with oil is antirheumatic and rubefacient; as a dicoction, it is stomachic, stimulant, carminative and diaphoretic. Sore throat, hoarseness and loss of voice are sometimes remedied by chewing a piece of ginger. Expressal juice from fresh ginger in gradually increasing doses in a strong diuretic. Raw and crystallized ginger is used as breath sweetener, an aid to digestion, and relief for flatulence; a cure for toothache and bleeding gums and as a strengthening agent for loose teeth and weak eyes (de Padua, Lugod and Pancho, 1987).

Betel (*Piper betle*)

Betel leaf is a diolcious, smooth, climbing vine, reaching a height of 2 to 4 meters. The upper leaves are usually oblong-elliptical, oblong-ovate, or ovate, 6 to 17 .5 centimeters long

3.5 to ten centimeters wide, 7 plinered, and smooth on both surfaces. The male spikes are subpendulous, slender,7 to 13-5 centimeters long, and 2 to 3.5 millimeters in diameter. The rachis is hairy. The two stamens are stalked, 0.75 to 1 millimeter long; and the anthers reniform. The female spikes, when mature, are red, fleshy, oblong to elongated oblong, 3 to 8 centimeters long, and 0.5 to 1 centimeter thick. The rachis is hairy, and the bracts stalkless, peltate, with a smooth disk, transversely oblong to suborbicular, and about 1 centimeter wide. The fruit is coalescing, fully embedded in the pulp and concresent with the rachis. The seeds are smooth, oblong to globose-obovoid, 2.25 to 2.6 millimeters long, and about 2 millimeters in diameter. The stigmas number 4 to 6, and, rarely, 3.

The chief constituent of the leaves is a volatile oil varying in the leaves from different countries and known as betel oil. It contains two phenols, betelphenol (chavibitol) and chavicol. Cadinene has also been found. Chavicol is a powerful antiseptic, twice as strong and isometric with eugenol. The characteristic odor of the leaves and oil is due to the chavicol. He says that the leaves also contain an alkaloid, arakene, with properties allied to cocaine. The betel oil contains also

terpene and sesquiterpene. The younger leaves contain much more essential oil, much diastase, and much more sugar than those which are older. The tannin content in both is the same. Betel oil is a light-yellow to dark-brown liquid, often aromatic, somewhat creosotelike in odor, and having a sharp burning taste. The specific gravity varies between 0.958 and 1.057. The Java betel contains betelphenol, llylpyrocatechol, ceniol, eugenol, methyl ether, and caryophyllene. Menthone is present also. The leaves contain vitamin C.

Betel leaf (also known locally as "ikmo", "buyo" or "hitsu") is cultivated throughout the Philippines and also occurs wild in most provinces of Luzon. It is also found in India to Malaya.

The Filipinos, Hindus, Malays, Siamese, Cambodians, Annamites, and Chinese (southern) use the leaves as a masticatory (the taste being warm, aromatic, and bitter), together with scraped areca nut and lime.

The leaves are stimulant, antiseptic, and sialogogue. They are carminative, astringent (juice of the leaves with oil), and aphrodisiac. The juice of the leaves is a valuable stomachic and

febrifuge. They are also useful expectorants. As an external medicine, the Filipinos use the fresh, crushed leaves as an antiseptic for cuts and wounds, and as a poultice for boils. A warm poultice of the leaves and oil (coconut) is applied on the chest of children in catarrhal and pulmonary affections, and is administered for congestion and other affections of the liver. The leaves are similarly employed as a resolvent for glandular swellings. The oil is used as a gargle, or as an inhalant in diphtheria.

In India the leaves, warmed and applied in layers, are used effectually for arresting the secretion of milk. The leaves are applied to the temples in headache for relieving pain. Chewing betel leaves early in the morning sweetens the breath, improves the voice, and removes all foulness of the mouth.

Concerning the medicinal use of the leaves, together with lime and betel nut, constitute a masticatory in general use among the Filipinos, who consider it a preservative of the teeth and a prophylactic against certain complaints of the stomach. The leaves, when greased with lard or sesame oil, are much used by Filipinos, as a carminative medicine applied to the abdomens of children suffering from gastric disorders. The juice of the leaves is regarded as a valuable stomachic.

In China, the roots , leaves, and fruits are considered to have carminative, stimulant, corrective, and prophylactic properties, and are used for the prevention and treatment of malaria.

A liquid extract is prescribed in catarrhal inflammations of the throat, larynx and brochi, and also in coughs, dyspnea, and indigestion. The roots with black pepper are used to produce sterelity in women. It is found that the oil is effective in inflammation of the throat, larynx, and bronchi, and as a gargle and inhalation in diphtheria (Quisumbing, 1978).

Staphylococcus aureus

S. aureus is a spherical bacterium which on microscopic examination appears in pairs, short chains, or bunched, grape-like clusters. These organisms are Gram-positive. Some strains are capable of producing a highly heat-stable protein toxin that causes illness in humans. The colonies formed by these bacteria are smooth, rounded and yellowish in color when grown in Mueller Hinton II agar at 20-25°C.

Staphylococcus aureus bacteria are often simply called "Staph" (pronounced "staff").

S. aureus is found on the skin and in the nostrils of many healthy individuals. These bacteria often give rise to minor superficial diseases, including the formation of pustules or boils in hair follicles. *S. aureus* infections are characterized by the presence of pus and formation of abscesses. In addition to skin pustules, boils, and carbuncles, *S. aureus* is responsible for impetigo, infections of wounds and burns (particularly in a hospital environment), breast abscesses, whitlow (inflammation of a finger or toe near the nail), osteomyelitis, bronchopneumonia, septicemia, acute endocarditis, food poisoning, and scalded skin syndrome. Scalded skin syndrome occurs in newborns and is due to infection by toxigenic strains of *S. aureus*. The toxins cause the skin to exfoliate, which leaves an appearance of having been scalded (Microsoft Encarta 98 Encyclopedia, 1998).

Staphylococcal food poisoning (staphyloenterotoxicosis; staphyloenterotoxemia) is the name of the condition caused by the enterotoxins which some strains of S. aureus produce.

The onset of symptoms in staphylococcal food poisoning is usually rapid and in many cases acute, depending on individual susceptibility to the toxin, the amount of contaminated food eaten, the amount of toxin in the food ingested, and the general health of the victim. The most common symptoms are nausea,

vomiting, retching, abdominal cramping, and prostration. Some individuals may not always demonstrate all the symptoms associated with the illness. In more severe cases, headache, muscle cramping, and transient changes in blood pressure and pulse rate may occur. Recovery generally takes two days. However, it is not unusual for complete recovery to take three days and sometimes longer in severe cases.

A toxin dose of less than 1.0 microgram in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when *S. aureus* populations exceed 100000 per gram.

Staphylococci exist in air, dust, sewage, water, milk, and food or on food equipment, environmental surfaces, humans, and animals. Humans and animals are the primary reservoirs. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50 percent or more of healthy individuals. This incidence is even higher for those who associate with or who come in contact with sick individuals and hospital environments. Although food handlers are usually the main source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *S. aureus*. Human intoxication is caused by

ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (60°C, 140°F, or above) or cold enough (7.2°C, 45°F, or below).

All people are believed to be susceptible to this type of bacterial intoxication; however, intensity of symptoms may Vary (http://vm.cfsan.fda.gov/, 1998).

About 20% - 30% of healthy people carry Staph bacteria in their noses at various times, without getting sick. And most of us begin to have Staph growing harmlessly on our bodies before we are one week old. Our fingers can carry Staph bacteria from one area of the body to another to cause infections in wounds or broken skin.

Most localized (small, nonserious) Staph skin infections can be treated by washing the skin with an antibacterial cleanser, applying an antibiotic ointment, and covering the skin with a clean dressing (http://KidsHealth.org/, 1998).

Bacillus subtilis

Bacillus subtilis are spore-forming rod shaped bacteria. Colonies are irregular and have a curled or hair-like structure sometimes called Medusa head.

Routes of infection of human beings are : through the skin, through respiratory tract, and through the alimentary canal. It is usually transmitted from lower animals rather than from other humans (Basic Microbiology, 1992).

Proteus vulgaris

Proteus vulgaris appear as straight or slightly curved rods 1 to 2.5 μ in length. It is included under the Enterobacteria and is related to but nevertheless distinct from the enteric bacilli.

They had been found to be associated with a variety of pathological conditions. Infections of the eye and ear, pluiritis and peritonitis and suppurative abscesses in many part of the body are among the many instances in which an etiological role is highly probable. As a production of cystitis and pylonephritis, it ranks next to *E. coli*.

It is found with some frequency in normal feces and often increases proportionately during or immediately after attacks of diarrheal disease caused by other organisms.

It is one of the most common bacteria in soil and contains decaying organic matter of animal origin.

Chapter 3

Research Design and Methodology

This experimental research aimed to determine the feasibility of dermal ointment utilizing ginger (*Zingiber officinale*) root and betel leaf (*Piper betle*) essential oils as the main ingredients.

The test organism used were the common bacteria which causes dermal infections, namely *Staphylococcus aureus, Bacillus subtilis* and *Proteus vulgaris.*

It was hypothesized that there would be no significant difference in the effectiveness of 35%% ginger root-35% betel leaf-30% petroleum jelly, 0% ginger root-70% betel leaf-30% petroleum jelly and, 70% ginger root-0% betel leaf-30% petroleum jelly treatments in terms of the zone of inhibiton they cause upon *Staphylococcus aureus Bacillus subtilis Proteus vulgaris* when compared with Dermalin and ether.

Materials and Equipment

The materials and equipment needed in this study are presented in Tables 1-5.

Table 1

Materials and Equipment for essential oil extraction

QTY	UNIT	EQUIPMENT/MATERIALS
	At least 250.00 g	Plant parts; ginger rhizomes; betel leaves
1		Osteorizer
1		Spatula
		Sieving cloth
10		Erlenmeyer flasks with stopper
1		Short term funnel
1		Long term funnel
1		Distilling flask
1		Condenser
1		Glass adaptor
		Cork borers
1		Mercury thermometer
		Distilled water
		Rubber tubing
10		Filter paper
10	Scoops	Anihydrous Sodium Sulfate
5	25 mL	Evaporating dishes
4		Iron stand with ring and clamps
2		Erlenmeyer flasks
		Diethyl ether
		Cotton plugs
1		Constant temperature H_2O
1		Separatory funnel

Table 2

Materials and Equipment needed for ointment preparation

QTY	UNIT	MATERIALS/EQUIPMENT
1	200 mL	Graduated cylinder
3	200 mL	Beaker
	150 mL	Essential oil: ginger and betel
1		Stirring rod
1		Heater
	300 mL	Petroleum jelly

Table 3

Materials and Equipment for subculture preparation

QTY	UNIT	MATERIALS/EQUIPMENT
4		Petri dishes
		Digital weighing scale
1		Inoculating loop
4		Agar slants of *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*
		Alcohol Burner
1		Autoclave

Table 4

Materials and Equiptment needed for inoculum and assay plate preparation

QTY	UNIT	MATERIALS/EQUIPMENT
1		Barium Sulfate Turbidity Standard
1		Oven
1		Inoculating loop
5	5 mL	Bunsen burner
5		Sterile pipets
1		Culture tubes
4	250 mL	Autoclave
1		Erlenmeyer flask
		Digital weighing scale

Table 5

Materials and equipment needed for Bauer-Kirby method of screening for presence of antibacterial activity; Antibacterial screening

QTY	UNIT	MATERIALS/EQUIPMENT
2	#42	Whattman filter paper
		Markers
	10mL	Distilled water
		Dermalin
		Puncher
		Incubator
20		Petri dishes

Extraction of essential oils from the local plants

Gathering of plant specimens. Active plant parts of each local plant were gathered. Intermediate rhizomes of ginger with no signs of diseases were used. Rhizomes were then pared and sliced into thin sections. Intermediate and fresh leaves of betel leaf with no signs of diseases were also gathered.

Each plant part was then washed thoroughly with running water and air-dried for 18 hours.

At least 300 grams of each active plant part were weighed and used for crude extraction.

Crude extraction of plant parts. The proportion used in this study was 1 gram plant part :2 cc water. Each of the plant part was processed in the osteorizer until thoroughly macerated, occasionally adding a small amount of the prepared volume of water. The processing of the plant part was continued until a thorough and uniform mixture was obtained.

The mixture was then placed in a stoppered Erlenmeyer flask and allowed to stand with occasional shaking for 36 hours. After 36 hours, the extract of the plant part was separated from the liquid portion using a fine sieve. The extract was placed in a stoppered Erlenmeyer flask, with remaining liquid from the

chopped plant part squeezed into the beaker until the chopped plant part was thoroughly dehydrated.

Distillation of crude extracts. The crude extract of the plant parts was used as distillants in this process. The distillant was placed in the distilling flask. Simple distillation for semi-volatile compounds was employed. The set-up made use of a distilling flask with boilintg chips connected to a condenser. The receiver flask was placed on an ice water bath.

The distillants were then placed in separate stoppered Erlenmeyer flasks.

Separation, drying, and purification of essential oils

Macro extraction of the essential oil from the distillants was used, as follows:

A dry separatory funnel was utilized, with the stopcock lubricated with stopcock grease.

Using an ordinary funnel, the distillant was then poured into the separatory funnel. The organic solvent, ether was then added and the ungreased glass stopper placed in position. Ether is both soluble to the water constituent of the distillant and to

the essential oil emulsions present, thus, distinct layers between water and oil were formed.

The separatory funnel was agitated for about three times and pressure was released by opening the stopcock for about three minutes. Formation of emulsion layers was avoided.

The stopper was rinsed with a few drops of organic solvent. Then, the separatory funnel was supported by an iron ring stand. The two liquids were allowed to separate into clear layers.

When the layers have settled, the glass stopper was removed, the stopcock opened and the lower layer , the water, allowed to flow into a beaker. The funnel was restoppered after the lower layer was disposed into the beaker completely.

The upper layer was funneled to an evaporating dish by pouring it through the mouth of the separatory funnel. A scoop of anihydrous sodium sulfate, a drying agent, was placed in a folded filter paper and placed in the funnel.

The ether in the ether-essential oil mixture was evaporated at 34.6 degrees C.

Purified essential oils after evaporation were placed in 25 mL E. flasks and refrigerated while not yet used.

Preparation of Ointment

The essential oil were mixed separately with petroleum jelly in low heat. The ointment was stored in a glass jar, sealed and kept inside the refrigerator.

Subculture preparation

The phase of the study detailed the transferring of pure culture to subculture agar plates. Bacterial cultures in subculture plates were used in the antibacterial assay.

Procurement of test organisms

Agar slants of *Staphylococcus aureus* and *Bacillus subtilis*, common Gram-positive pathogens and *Proteus vulgaris* , a Gram-negative bacterium were obtained from Southeast Asian Fisheries Development Center.

Autoclave settings

The standard sterilization settings in using the autoclave was 115 lb. Per sq. in. (psi) for steam pressure, 121 degrees C or 250 degrees F for temperature for 15 minutes.

Preparation of the petri dishes

Three petri dishes, one for each test organism, were thoroughly sterilized using the autoclave.

Culture media preparation

About 6 grams of agar were weighed in a digital weighing scale and mixed with 150 mL of distilled water. The agar was then allowed to melt. The prepared rehydrated agar was then placed in a beaker- allowing it to boil for about one minute by placing it in a hot plate- and then sterilization followed.

Using the tongs, about 12 to 15 mL of the melted agar medium were poured in each of the sterilized petri dishes. Strict aseptic methods were observed.

The agar was then allowed to harden for 15 to 20 minutes but not more than 20 minutes. The agar plates were sterilized in the autoclave using the above mentioned considerations.

Streak inoculation of bacteria on agar plates

After autoclaving, the agar plates were oven-dried to 100 degrees C and allowed to harden and then readied for streak inoculation.

Aseptic methods were strictly observed so as not to contaminate the specimen. Disposable inoculating loops were used, one for each bacteria.

The cap of the test specimen container was then removed. A thin loopful of the specimen was removed. The container's cap was replaced at once.

With the agar plate side cover down the work surface, the plate containing the agar was lifted and held in one hand during the inoculating step. Approximately one-fourth of the surface was then streaked with the test material on the loop.

The plate was rotated to quarter turn and streaking be done again, overlapping the originally streaked area.

The inoculated agar plates were then incubated in the oven at 37 degrees C for 24 hours. Colonies in each agar plate were then formed after the end time.

Inoculum and assay plate preparation

Preparation of the barium sulfate turbidity standard. A 0.5 McFarland barium sulfate turbidity standard was prepared by coding 0.5 mL of 0.043 M $BaCl_2$ [(1.75%wt/vol) $BaCl_2$%$2H_2O$] to 99.5 mL of 0.3 M H_2SO_4 (11% wt/vol). This standard was then mixed thoroughly.

Preparation of the chicken broth . 25 mL of chicken broth was then cooked according to the proportion; 2 g powder is to 1 L H_2O.

Preparation of the test organisms. Five well-isolated colonies from prepared plate culture (colonies should not be less than 2 mm in diameter) were selected and transferred with an inoculating loop to the test tube containing 5 mL of chicken broth. The tube culture with stopper was mixed thoroughly through shaking, then its turbidity was compared with the Barium Sulfate Standard until it was reached. The turbidity of the broth culture was adjusted for it to be equivalent to the barium sulfate standard. If dilution was needed, sterile broth was added to the test tube.

About 100 mL of sterilized Mueller Hinton II agar were cooked using the given proportion (37 g: 1 L). A sufficient amount of agar medium was then allowed to melt and cool down to 48 degrees C. The melted Agar was then distributed among three labeled Erlenmeyer flasks, each for each test organism. Inoculum was then added to the agar to give it a concentration of about 1%. The seeded agar was then mixed thoroughly.

Preparation of assay plates. 700 mL of sterilized Mueller Hinton II agar were prepared, using the above mentioned proportion.

Fifteen petri dishes were prepared for three test organisms with five replicates each. Twenty one mL of cooked agar were placed in each of the petri dishes. The agar was distributed evenly in the plate and allowed to harden. After hardening, the base plates were autoclaved for 15 minutes under the above mentioned conditions. They were then be oven dried with the temperature of 100° C for one hour.

After oven drying, the agar in the base plates were allowed to harden. Petri dishes were then labeled as BnRx; n as the number of bacteria; and x as the replicate number. Five replicates were used per test organism.

Using a 5 mL pipette, four mL of seeded agar of a particular bacteria were placed in the designated petri dishes.

Antibacterial screening of the ointment

Preparation of the antibacterial discs. About 150 discs were punched out from pieces of Whattman number 42 filter paper, about 6 mm in diameter each. These were wrapped in a piece of clean paper placed in the autoclave for sterilization.

Using the forceps, 25 discs were placed in the Erlenmeyer flask of a particular treatment. Twenty five discs were soaked in Ether for the negative control, and the other 25 in Dermalin for the positive control.

The discs were soaked in the treatments for 24 hours.

Labeling. The agar plates were separated into three groups for the three bacteria. In each treatment, five replicates were used. Each treatment impregnated in the filter paper was designated to a specific treatment concentration.

Treatments 4 and 5 were the positive and negative controls.

An arrow was drawn under the petri dish to distinguish one treatment from the other, in order of their precedence.

Disc agar diffusion method and application. Using sterile forceps and observing strict aseptic precautions, the membrane discs corresponding to the treatment as shown by the arrows on the bottom of the plates were placed on the inoculated surface. The discs were deposited so that the centers are at least one inch apart.

After the discs were applied, the plates were inverted and placed in a 37° C incubator. The agar plates were incubated for 24 hours at 37° C. A confluent mat of growth was obtained after the 24-hour incubation.

Data Gathering and Statistical Analyses

Measuring the diameter of zones

The diameter of zones of complete inhibition including the diameter of the discs was measured to the nearest mm, using a straight rule. A diameter zone of inhibition of more than 6 mm indicated a positive result.

Statistical analyses of results

The One-way Analysis of Variance (ANOVA), set at 0.05 level of significance was employed to determine the significant difference in the effectivity of the different concentrations of ginger root and betel leaf. The Scheffe test, also at 0.05 level of significance, was utilized as a post-hoc multiple comparison test.

Chapter 4

Results and Discussion

This experiment research aimed to determine the feasibility of dermal ointment utilizing ginger (*Zingiber officinale*) root and betel (*Piper betle*) leaf extracts as the main ingredients.

The test organism used were the common bacteria which causes dermal infections, namely *Staphylococcus aureus, Bacillus subtilis* and *Proteus vulgaris.*

It was hypothesized that there is no significant differences in the effectiveness of 35% ginger root-35% betel leaf-30% petroleum jelly, 0% ginger root-70% betel leaf-30% petroleum jelly and, 70% ginger root-0% betel leaf-30% petroleum jelly treatments in terms of the zone of inhibition they cause upon *Staphylococcus aureus, Bacillus subtilis* and *Proteus vulgaris.*

Feasibility of Dermal Ointment from Ginger Root and Betel Leaf

The dermal ointment from the essential oil of ginger root and betel leaf showed effectiveness as shown by their mean zones of inhibition caused upon the test organisms.

The ginger ointment showed the following mean zones of inhibition against *S.aureus, B. subtilis,* and *P. vulgaris*: 7.4 mm, 7.8 mm, and 7.8 mm, respectively.

Table 1 shows the data.

The betel-ginger ointment showed the following mean zones of inhibition against *S. aureus*, *B. subtilis*, and *P. vulgaris*: 8.4 mm, 9.0 mm, and 7.2 mm, respectively.

Table 1 shows the data.

The betel ointment showed the following mean zones of inhibition against *S. aureus*, *B. subtilis*, and *P. vulgaris*: 7.4 mm, 7.8 mm, and 7.6 mm, respectively.

The negative control (Ether) showed no zones of inhibition upon *S. aureus*, *B. subtilis*, and *P. vulgaris*.

Table 1 shows the data.

The positive control (Dermalin) showed the following mean zones of inhibition against *S. aureus*, *B. subtilis*, and *P. vulgaris*: 7.6 mm, 7 10.2 mm, and 8.0 mm, respectively.

Table 1 shows the data.

These results show that the dermal ointment mentioned was feasible.

Effectiveness of the Ointment Treatments on *Staphylococcus aureus*

Diameter zones of inhibition, which were measured to the nearest millimeter, formed by the five treatments on *Staphylococcus aureus* differed significantly, as reflected by $F(20) = .000$, $p < .05$.

Table 1

Mean diameter zones of inhibition formed by the treatments on the three test organisms

Treatment/Test Organism	*S.aureus*	*B.subtilis*	*P. vulgaris*
T_1: Ginger ointment	7.4	7.8	7.8
T_2: Betel-Ginger ointment	8.4	9.0	7.2
T_3: Betel ointment	7.4	7.8	7.6
T-: Ether	0	0	0
T+: Dermalin	7.6	10.2	8.0

Table 2 shows the data.

The Scheffe test showed that the mean differences in zones of inhibition of *S. aureus* were significant between 70% ginger-0% betel-30% petroleum jelly and ether, between 35% ginger-35% betel-30% petroleum jelly and ether, between 0% ginger-70% betel-30% petroleum jelly and ether, and between ether and Dermalin.

Table 3 shows the data.

Table 2

One-Way ANOVA of the differences in the effectiveness of the different extracts against the test organisms

Category	Source of Variation	Sum of Squares	df	Mean Square	F	Significance
Zones of Inhibition of *B. subtilis*	Between groups	322.560	4	80.640	42.000	.000
	Within groups	38.400	20	1.920		
	Total	360.960	24			
Zones of Inhibition of *S. aureus*	Between groups	235.840	4	58.960	62.723	.000
	Within groups	18.800	20	.940		
	Total	254.640	24			
Zones of Inhibition of *P. vulgaris*	Between groups	240.560	4	60.140	250.583	.000
	Within groups	4.800	20	.240		
	Total	245.360	24			

Table 3

Scheffe test as post-hoc multiple test for *S. aureus* after the One-Way ANOVA in Table 2

	Treatment	Treatment	Mean Difference	Std. Error	Significance
S. aureus	70%Ginger-0%Betel-30%Petroleum Jelly	35%Ginger-35%Betel-30%P. jelly	-1.00	.310	.067
		70%Betel-0%Ginger-30%P. jelly	.000	.310	1.000
		Ether	7.40*	.310	.000
		Dermalin	-.20	.310	.980
	35%Ginger-35%Betel-30% P. jelly	0%Ginger-70%Betel-30%P. jelly	1.00	.310	.067
		Ether	8.40*	.310	.000
		Dermalin	.80	.310	.197
	70%Betel-0%Ginger-30%Petroleum jelly	Ether	7.80*	.310	.000
		Dermalin	-.20	.310	.980
	Ether	Dermalin	-10.20*	.310	.000

*The mean difference is significant at the .05 level.

Effectiveness of the Ointment Treatments on *Bacillus subtilis*

Diameter zones of inhibition, which were measured to the nearest millimeter, formed by the five treatments on *Bacillus subtilis* differed significantly, as reflected by $F(20) = .000$, $p < .05$.

Table 2 shows the data.

The Scheffe test showed that the mean differences in zones of inhibition of *B. subtilis* were significant between 70% ginger-0% betel-30% petroleum jelly and ether, between 35% ginger-35% betel-30% petroleum jelly and ether, between 0% ginger-70% betel-30% petroleum jelly and ether, and between ether and Dermalin.

Table 4 shows the data.

Effectiveness of the Ointment Treatments on *Proteus vulgaris*

Diameter zones of inhibition, which were measured to the nearest millimeter, formed by the five treatments on *Proteus vulgaris* differed significantly, as reflected by $F(20) = .000$, $p < .05$.

Table 2 shows the data.

The Scheffe test showed that the mean differences in zones of inhibition of *P. vulgaris* were significant between 70% ginger-0% betel-30% petroleum jelly and ether, between 35% ginger-35% betel-30% petroleum jelly and ether, between 0% ginger-70% betel-

Table 4

Scheffe test as post-hoc multiple test for *B. subtilis* after the One-Way ANOVA in Table 2

	Treatment	Treatment	Mean Difference	Std. Error	Significance
B. subtilis	70%Ginger-0%Betel-30%Petroleum Jelly	35%Ginger-35%Betel-30%P. jelly	-1.20	.876	.758
		70%Betel-0%Ginger-30%P. jelly	.000	.876	1.000
		Ether	7.80*	.876	.000
		Dermalin	-2.40	.876	.154
	35%Ginger-35%Betel-30% P. jelly	0%Ginger-70%Betel-30%P. jelly	1.20	.876	.758
		Ether	9.00*	.876	.000
		Dermalin	-1.20	.876	.758
	70%Betel-0%Ginger-30%Petroleum jelly	Ether	7.80*	.876	.000
		Dermalin	-2.40	.876	.154
	Ether	Dermalin	-10.20*	.876	.000

*The mean difference is significant at the .05 level.

308 petroleum jelly and ether, and between ether and Dermalin. 46

Table 5 shows the data.

Table 5

Scheffe test as post-hoc multiple test for P. vulgaris after the One-Way ANOVA in Table 2

	Treatment	Treatment	Mean Difference	Std. Error	Significance
P. vulgaris	70%Ginger-0%Betel-30%Petroleum Jelly	35%Ginger-35%Betel-30%P. jelly	.60	.613	.913
		70%Betel-0%Ginger-30%P. jelly	.20	.613	.999
		Ether	7.80*	.613	.000
		Dermalin	-.20	.613	.999
	35%Ginger-35%Betel-30% P. jelly	0%Ginger-70%Betel-30%P. jelly	-.40	.613	.979
		Ether	7.20*	.613	.000
		Dermalin	-.80	.613	.788
	70%Betel-0%Ginger-30%Petroleum jelly	Ether	7.60*	.613	.000
		Dermalin	-.40	.613	.979
	Ether	Dermalin	-8.00*	.613	.000

*The mean difference is significant at the .05 level.

Chapter 5

Summary, Conclusions, and Recommendations

Summary

This study aimed to determine the feasibility of producing dermal ointment using ginger (*Zingiber officinale*) root and betel (*Piper betle*) leaf extracts as the main ingredients.

Furthermore, it aimed to determine the effectivity of the ointment product against *Staphylococcus aureus*, *Bacillus subtilis*, and *Proteus vulgaris*.

Specifically, it sought answers to the following questions:

1. Is the dermal ointment with (a) ginger root and (b) betel leaf extracts as main ingredients feasible?

2. Is the dermal ointment with (a) ginger root, (b) betel leaf extracts effective against (1) *Staphylococcus aureus*, (2) *Bacillus subtilis*, (3) *Proteus vulgaris*?

3. Is there a significant difference in the effectivity of the (a) 35% ginger root-35% betel leaf-30% petroleum jelly (b) 0% ginger root-70% betel leaf-30% petroleum jelly and, (c) 70% ginger root-0% betel leaf-30% petroleum jelly treatments effective in terms of the zones of inhibition they cause upon

(1) *Staphylococcus aureus*, (2) *Bacillus subtilis* and (3) *Proteus vulgaris*?

Based on the problems given, it was hypothesized that there would be no significant difference in the effectivity of (a) 35% ginger root-35% betel leaf-30% petroleum jelly, (b) 0% ginger root-70% betel leaf-30% petroleum jelly and, (c) 70% ginger root-0% betel leaf-30% petroleum jelly treatments in terms of the zones of inhibition they caused upon (1) *Staphylococcus aureus*, (2) *Bacillus subtilis* and (3) *Proteus vulgaris* compared with a commercial ointment and ether.

Findings

This study was able to establish the following findings based on the data gathered:

1. All the ointment treatments were able to inhibit the test organisms, the Gram-positive pathogens *Staphylococcus aureus* and *Bacillus subtilis*; and Gram-negative bacteria *Proteus vulgaris*. Diameter zones of inhibition were the parameters used in screening for the presence of antibacterial activity in the ointment treatments.

2. The dermal ointment from ginger roots and betel leaf was effective against the test organisms.

3. The One-way Analysis of Variance proved that for both test organisms, the zones of inhibition formed by the ointments from the different treatments differed significantly among each other. The Scheffe test, on the other hand, proved that there were no significant difference in the effectiveness of all treatments and the commercial ointment Dermalin, and a significant difference in the effectiveness of all treatments and the control.

Conclusions

From the findings of this study, the following conclusions were drawn:

The production of an antibacterial dermal ointment from ginger (*Zingiber officinale*) and betel leaf (*Piper betle*) essential oil was feasible. The product was tested and compared with a commercial ointment Dermalin.

The ointment products in different treatment concentrations were effective against the three pathogenic bacteria.

Against *S. aureus*, the 35% ginger-35% betel-30% petroleum jelly, caused the greatest mean diameter zone of inhibition. It was therefore, the most effective concentration.

In the two other bacteria, Dermalin caused the greatest mean diameter zone of inhibition. It was therefore, the most effective ointment.

No significant differences in the effectiveness of the dermal ointment from the different treatments and the commercial ointment against the three test bacteria were observed. The dermal ointment from ginger and betel leaf, therefore, was as effective as the commercial ointment.

Recommendations

Basing on the results and conclusions we have obtained from this feasibility study, the researchers seek to recommend the following:

That if other feasibility studies conducted along this line were pursued in the future, the number of replicates be increased to ensure more accuracy in the attainment of data.

That the essential oils of some other local medicinal plants be extracted and tested for antibacterial activity on the same bacterial strains used herein, or more, depending on the researchers' preference.

That if significant results were achieved in the initial testing for antibacterial activity inn the said plants, the feasibility of their becoming dermal ointments must also be pursued in order for the study's results to be put to more practical uses.

That other future researchers perform the study again to further verify the data and results obtained from this study.

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